Attachment 4

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Immunology

Cholera toxin B subunit: An efficient transmucosal carrier–delivery system for induction of peripheral immunological tolerance

(oral tolerance)

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ABSTRACT **Oral administration of antigens, including allergens and autoantigens, may be an efficient way to prevent diseases associated with untoward immune responses to self- and non-self-antigens. However, this approach has met with limitations because it usually requires repeated administrations of large doses of antigen and is less efficient in an already immune host, and the effect is of short duration. We report that a single oral administration of minute amounts of particulate or soluble antigen coupled to the B subunit of cholera toxin (CTB) can markedly suppress systemic immune responses in naive and in systemically immune animals. Both early (2–4 hr) and late (24–48 hr) delayed type-hypersensitivity reactivities were strongly suppressed after feeding a single dose of CTB-conjugated antigen. Serum antibody responses were also decreased, although moderately, after oral administration of CTB-conjugated antigen. This strategy of tolerance induction, based on oral administration of small amounts of antigens conjugated to a mucosa-binding molecule, may find broad applications for preventing or abrogating untoward immune responses.**

Oral administration of antigens is a long-recognized method for inducing peripheral immunological tolerance (1, 2) and has been proposed as a means to prevent or treat allergic reactions (3, 4), Rh alloimmunization (5), and experimental autoimmune diseases (6–13). Efforts to develop optimal tolerogenic formulations based on this strategy have been stimulated by recent studies reporting beneficial effects of oral administration of antigens in patients with autoimmune diseases (14–16).

Although oral administration of antigens offers a convenient way to induce systemic tolerance, its therapeutic potential has been seriously limited. Indeed, unless tolerogens are administered repeatedly and in large doses, tolerance is usually modest and of short duration (17, 18), being rather difficult to induce in an already-immune host (19–22).

We now report that a single oral administration of a small dose of a soluble or particulate antigen conjugated to the B subunit of cholera toxin (CTB), rather than abrogating systemic tolerance to conjugated antigens, as generally assumed (23–25), can profoundly enhance it in naive as well as in immune animals.

MATERIALS AND METHODS

Animals. BALB/c and C57BL/6J female mice, 6–8 weeks old at the start of experiments, were used.

Antigens. Purified human γ-globulin (HGG) was purchased from Pharmacia. Sheep red blood cells (SRBCs) and horse red blood cells (HRBCs) were obtained from the National Institute of Veterinary Medicine (Håtunaholm, Sweden). Cholera toxin (CT) was obtained from List Biological Laboratories (Campbell, CA).

Preparation of CTB-Conjugated Antigens. CTB was purified from the culture supernatant of a mutant strain of *Vibrio cholerae* deleted of the CT genes and transfected with a plasmid encoding CTB (26, 27).

To facilitate coupling to CTB, SRBCs and HRBCs were first derivatized with monosialoganglioside (GM1). A solution of GM1 (Sigma) [300 nmol/ml in phosphate-buffered saline (PBS)] was added to packed red cells at a ratio of 1:2 (vol/vol) and the mixture was incubated for 2 hr at 37°C with shaking. After three washes with PBS, GM1-coated red cells were resuspended in PBS. CTB was added to the cell suspension in molar excess to the cell-bound GM1 (50 μg of CTB per 5×10^9 GM1-SRBCs per ml). After 2 hr at 37°C, the erythrocytes were washed twice with PBS to remove unbound CTB. A solid-phase hemadsorption assay using GM1 immobilized on plastic wells was employed to ascertain that the CTB molecules (pentamers) had bound to GM1-coupled SRBCs or HRBCs and were still able to bind additional GM1 molecules.

HGG was covalently conjugated to CTB by using *N*-succinimidyl[3-(2-pyridyl)dithio]propionate (SPDP) as bifunctional coupling reagent (28), essentially according to the supplier's instructions (Pharmacia). In brief, CTB and HGG were separately derivatized with SPDP at molar ratios of 1:5 and 1:10, respectively. After reduction and purification by Sephadex G-25 chromatography, the SPDP-derivatized HGG was incubated with SPDP-derivatized CTB (at ratios of 1:1, 3:1, and 9:1) for 16 hr at 23°C. The resulting CTB–HGG conjugates were purified by gel filtration through Sephacryl S-300 and shown to contain GM1-binding capacity and to retain both CTB and HGG serological reactivities by means of a solid-phase ELISA using GM1 as capture system (29) and enzyme-labeled antibodies to CTB and HGG as detection reagents.

Systemic Immunization Protocols. Mice were primed with SRBCs or HRBCs by injecting the left rear footpad with 40 μl of pyrogen-free saline containing 10^7 red blood cells. Five to 7 days after priming, animals were challenged by injecting the right rear footpad with pyrogen-free saline containing 10^8 SRBCs or HRBCs. Separate groups of mice were primed by subcutaneous injection of heat-aggregated (63°C, 30 min) HGG (500 μg) emulsified in Freund's complete adjuvant (Difco). One week later, animals were challenged by injecting each of the rear footpads with 0.5 mg of HGG in saline.

For control purposes, separate groups of unprimed animals were challenged similarly with SRBCs, HRBCs, or HGG.

Oral Tolerization Protocols. At various times before or after systemic priming with red cells, mice were given a single dose

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Abbreviations: CT, cholera toxin; CTB, CT B subunit; GM1, monosialoganglioside; SRBC, sheep red blood cell; HRBC, horse red blood cell; HGG, human γ-globulin; DTH, delayed-type hypersensitivity.

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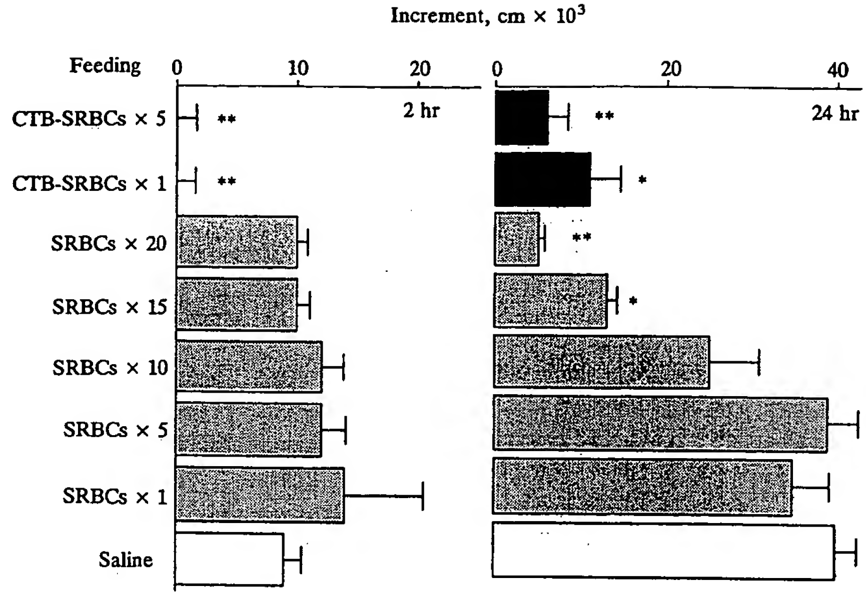

FIG. 1. Suppression of systemic DTH reactions after oral administration of CTB-conjugated SRBCs. Groups of BALB/c mice (six or eight per group) were fed unconjugated or CTB-conjugated SRBCs given as single or repeated doses. One week after the last oral administration, animals were primed systemically with SRBCs. DTH responses were elicited 5 days later by footpad injection of SRBCs and determined by standard footpad thickness measurement. Data are expressed as mean specific footpad thickness increment (with SD indicated by error bars) which was calculated at 2 hr (*Left*) and at 24 hr (*Right*) after challenge with SRBCs. Negative values were adjusted to zero. Asterisks denote significant differences with saline-fed animals (*, $P < 0.01$; **, $P < 0.001$; Mann–Whitney U test).

or daily consecutive doses of unconjugated or CTB-conjugated red cells. Each dose consisted of 2.5×10^9 erythrocytes in 0.5 ml of PBS given by the intragastric route.

For induction of tolerance to HGG, mice were given a single oral dose of CTB-conjugated or unconjugated HGG, 7 days before systemic priming. Doses of 75 μg, 1 mg, or 5 mg of unconjugated HGG and 8.5, 25, or 75 μg of HGG conjugated to CTB were administered in 0.5 ml of 0.35 M $NaHCO_3$ by gastric intubation.

Evaluation of Delayed-Type Hypersensitivity (DTH) Reactions. Thickness of the rear footpads was measured with a dial gauge caliper (Oditest, Essen, Germany) before and 2, 4, 24, and 48 hr after systemic challenge. The intensity of DTH reactions was determined in each animal by subtracting the value obtained before challenge from those obtained at various times after challenge. Specific footpad thickness increment was calculated by subtracting the background swelling at 2, 4, 24, and 48 hr after challenge of unprimed control animals from the swelling of primed mice to provide the net footpad response (30).

***In Vitro* Lymphocyte Proliferative Responses.** Single-cell suspensions were prepared from pooled spleen and popliteal lymph nodes 1 week after footpad priming with SRBCs. Mononuclear cells were cultured in quadruplicate sets for 3 days in flat-bottom microplates at 10^5 cells per well in 0.2 ml of Iscove's medium (Gibco) with 5% fetal bovine serum and with either SRBCs (5×10^8), concanavalin A (Sigma) (0.2 μg), or no stimulus. Cultures were incubated during the last 16 hr with [methyl-^{3}H]thymidine (Amersham) (1 μCi/well; 1 μCi = 37 kBq). Results are expressed as the stimulation index (SI), defined as the ratio of the mean radionucleotide incorporation of SRBC-stimulated cultures divided by that of unstimulated cultures.

Serology. Levels of serum antibodies to SRBCs and HRBCs were determined by direct and indirect hemagglutination assays using heat-inactivated sera. Serial 2-fold serum dilutions were prepared in U-bottom microwells, and an equal volume (50 μl) of a suspension of 0.5% red cells was added. After 2 hr at room temperature and overnight at 4°C, wells were examined for hemagglutination. To detect nonhemag-

Table 1. Oral administration of CTB-conjugated red cells induces antigen-specific, long-lasting suppression of systemic DTH responses and is effective in systemically immune animals

Oral tolerogen (day)*	Systemic immunogen†	Specific thickness increment,‡ cm × 10³ (% inhibition) 2 hr	24 hr
CTB-SRBCs × 1 (−7)	SRBCs	−3 ± 3.8 (126; $P < 0.001$)	2 ± 0.6 (96; $P < 0.001$)
CTB-HRBCs × 1 (−7)	SRBCs	9 ± 3.5 (10)	36 ± 6.1 (20)
Saline	SRBCs	10 ± 2.4	45 ± 5.7
CTB-HRBCs × 1 (−7)	HRBCs	−4 ± 5.6 (147; $P < 0.001$)	5 ± 2.1 (8.4; $P < 0.01$)
CTB-SRBCs × 1 (−7)	HRBCs	7 ± 1.6 (22)	34 ± 4.2 (−9)
Saline	HRBCs	9 ± 1.2	31 ± 3.5
CTB-SRBCs × 1 (−56)	SRBCs	0 ± 1.9 (100; $P < 0.01$)	17 ± 6.5 (70; $P < 0.05$)
SRBCs × 15 (−56)	SRBCs	8 ± 3.4 (36)	6 ± 3.3 (93; $P < 0.01$)
Saline	SRBCs	11 ± 3.6	56 ± 7.0
CTB-SRBCs × 1 (0)	SRBCs	3 ± 1.1 (88; $P < 0.01$)	3 ± 1.0 (91; $P < 0.01$)
CTB-SRBCs × 1 (+4)	SRBCs	−5 ± 3.3 (122; $P < 0.001$)	−9 ± 7.1 (127; $P < 0.001$)
SRBCs × 1 (0)	SRBCs	19 ± 2.6 (21)	22 ± 4.4 (33)
SRBCs × 1 (+4)	SRBCs	20 ± 4.9 (17)	26 ± 15 (21)
Saline	SRBCs	24 ± 2.3	33 ± 3.6

*Indicates the day of administration of a single oral dose or of the last of 15 oral doses of unconjugated or CTB-conjugated red cells.
†Administered by footpad injection on days 0 and +5.
‡Mean ± SD determined on groups of six to eight mice. Where significant, differences with saline-fed animals are noted (Wilcoxon rank test).

glutinating IgG antibodies, affinity-purified goat antibodies to mouse IgG Fc fragment (Southern Biotechnology Associates) were added (0.25 μg per well) and the plates were reincubated. The antibody titer was defined as the reciprocal of the highest dilution of serum causing hemagglutination before and after facilitation with anti-mouse IgG.

Serum IgM and IgG antibody levels to HGG were determined by solid-phase ELISA using polystyrene-bound HGG as capture system and horseradish peroxidase-conjugated, affinity-purified, isotype-specific goat anti-mouse Ig antibodies (Southern Biotechnology Associates) as detection reagents.

RESULTS

Suppression of DTH by Oral Administration of CTB-Conjugated Antigens. Mice were fed SRBCs alone, CTB-SRBCs, or saline solution 1 week before primary systemic immunization with SRBCs. Five days after systemic priming, DTH responses to footpad challenge with SRBCs were recorded. In mice fed a single dose of SRBCs conjugated to the mucosa-binding molecule CTB, DTH reactivity was abrogated or markedly reduced, at all times examined (Fig. 1). Thus, 2 hr after challenge with SRBCs—i.e., at a time corresponding to the early reaction of a DTH response (30)—footpad swelling was absent in mice previously fed one dose of CTB-SRBCs (Fig. 1). Further, the late DTH response, which in mice peaks around 24 hr after challenge, was strongly suppressed in animals fed CTB-SRBCs compared with saline-fed animals (Fig. 1). Comparable results were obtained in mice fed a single dose or up to five doses of CTB-SRBCs. In contrast, feeding mice 1 or up to 10 daily consecutive doses of unconjugated SRBCs given alone or with free CTB, or of GM1-derivatized SRBCs alone (without CTB), had no appreciable effects on subsequent DTH reactivity. Daily oral administration of SRBCs 5 days per week for 3–4 weeks (i.e., 15–20 doses) was required to suppress the 24-hr DTH reaction to a level comparable to that achieved by a single dose of CTB-SRBCs, while as many as 20 consecutive feedings with unconjugated SRBCs had no effect on the early (2 hr) swelling reaction (Fig. 1). Moreover, early as well as late DTH reactions to footpad injection with SRBCs were still suppressed in mice fed 8 weeks earlier with a single dose of CTB-SRBCs, whereas mice fed 15 consecutive doses of unconjugated SRBCs, the last dose having been given 8 weeks before systemic priming with SRBCs, had reduced late DTH responses but intact early swelling reactivity (Table 1).

Animals fed CTB-SRBCs developed normal DTH reactivity to HRBCs (Table 1). Feeding animals CTB-HRBCs abrogated early and late DTH reactivities to HRBCs but not to SRBCs (Table 1). These observations demonstrate that suppression of early and late DTH responses by oral administration of CTB-conjugated red cells is antigen specific.

In another set of experiments, mice were primed with SRBCs injected in the left rear footpad. Animals were fed one dose of CTB-conjugated or unconjugated SRBCs given at the time of priming or 4 days later. Seven days after systemic priming, mice were challenged in the right rear footpad with SRBCs. Whereas mice fed SRBCs alone developed DTH responses comparable to those seen in saline-fed animals, mice fed CTB-SRBCs given at the time of or 4 days after systemic priming had virtually no early and late DTH reactivities to SRBCs (Table 1). Thus, oral administration of CTB-SRBCs can induce long-lasting antigen-specific suppression of systemic DTH responses in naive animals and can abrogate these responses in systemically immune animals.

To determine whether mucosal administration of CTB-conjugated antigens would also suppress DTH reactions to soluble antigens, mice were fed one dose of CTB-conjugated or unconjugated HGG. Animals were then systemically primed with HGG, and DTH reactions to footpad challenge with HGG were monitored. Feeding mice as much as 1 mg of unconjugated HGG had no effect on DTH reactivity to HGG (Fig. 2). Feeding mice 5 mg of HGG partly suppressed the late but not the early DTH response to HGG. In contrast, a single oral administration of a >500-fold lower dose (8.5 μg) of CTB-HGG had comparable effects, suppressing partly the late but not the early DTH response, and in mice fed as little as 75 μg of CTB-HGG, both early and late DTH reactions to HGG were abolished (Fig. 2). Feeding mice with a comparable amount (75 μg) of unconjugated HGG did not affect systemic DTH reactivity to HGG (data not shown).

Suppression of Lymphocyte Proliferative Responses After Feeding CTB-Conjugated Antigen. Compared with lymphocytes from saline-fed animals or from animals given one oral dose of unconjugated SRBCs, cells from mice given one dose of CTB-SRBCs had decreased proliferative responses to

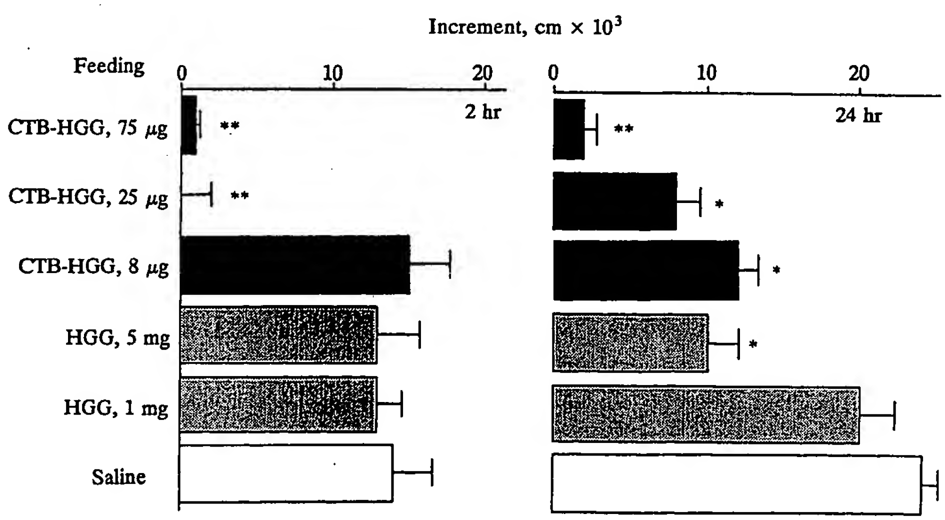

FIG. 2. Suppression of systemic DTH reactions after oral administration of CTB-conjugated HGG. Groups of C57BL/6J mice (8–10 per group) were fed one dose of unconjugated or CTB-conjugated HGG given 1 week before systemic sensitization with HGG. One week after sensitization, DTH reactions were elicited with HGG injected in the rear footpads. Specific increments in footpad swelling were determined after subtracting the mean background footpad thickness determined on a group of 10 unprimed but similarly challenged control animals. Data are expressed as in Fig. 1.

Table 2. Suppression of antigen-induced lymphocyte proliferative responses after oral administration of CTB-conjugated SRBCs

	Stimulation index (% inhibition)	
Oral tolerogen	SRBC	Con A
CTB-SRBCs	0.98 ± 0.36 (85; $P < 0.01$)	119 ± 32
SRBCs	3.92 ± 2.95 (38; $P > 0.05$)	129 ± 23
Saline	6.35 ± 2.47	86 ± 35

Mice were fed a single dose of unconjugated or CTB-conjugated SRBCs given 1 week prior to systemic priming with SRBCs. Proliferative responses of pooled lymph node and spleen cells were determined for six animals per group in cultures initiated 1 week after priming and are expressed as mean stimulation index ± SD.

SRBCs *in vitro* (Table 2). Proliferative responses to concanavalin A were comparable in all animal groups.

Suppression of Systemic Antibody Responses After Feeding CTB-Conjugated Antigen. Serum IgM and IgG antibody responses to systemically injected SRBCs were decreased by a factor of ≈5 in mice previously fed a single dose of CTB-SRBCs compared with saline-fed control animals (Table 3). Daily oral administrations of unconjugated SRBCs for 3 weeks were required to suppress these responses to levels comparable to those obtained after a single oral dose of CTB-SRBCs. Further, a single oral dose of CTB-SRBCs given to mice at the time of (day 0) or 4 days after systemic priming with SRBCs—i.e., at a time when hemagglutinating antibodies were not yet detectable in serum—suppressed primary serum IgM anti-SRBC antibody responses and also reduced, by a factor of ≈5, secondary serum IgG antibody responses evoked by systemic challenge with SRBCs. In contrast, feeding SRBC-immune animals with the same dose of unconjugated SRBCs had no effect on such serum antibody responses. In mice fed HGG conjugated to CTB, on the other hand, serum IgM and IgG antibody responses to HGG were only modestly reduced (by ≈50%) compared with control mice fed saline only and with mice fed as much as 5 mg of unconjugated antigen (data not shown).

CT Abrogates CTB-Induced Oral Tolerance. Feeding mice one dose of SRBCs conjugated to CT not only failed to suppress early and late DTH responses to SRBCs (Table 4) but was in fact effective at priming animals for systemic DTH responses to SRBCs (data not shown). Mice fed free CT together with CTB-SRBCs developed normal if not enhanced DTH (Table 4) and serum antibody (data not shown) responses to SRBCs. Feeding mice as much as 500 μg of free CTB together with CTB-SRBCs had no effect on suppression of DTH reactivity to SRBCs (Table 4).

DISCUSSION

It has been assumed that among nonliving immunogens, only those with mucosa-binding and possibly also immunostimulatory properties can induce local and systemic immune responses without inducing systemic immunological tolerance, when administered by a mucosal route (25). A notable example is CT, one of the most potent mucosal immunogens (31–33), which, when administered orally with an unrelated antigen, can also prevent induction of systemic tolerance to that antigen (24). These unusual features can be partly explained by the ability of CTB to bind avidly to GM1 on cell surfaces, and to the ADP-ribosylating action of the toxic A subunit of CT (33, 34). Based on these observations, mucosal administration of antigen coupled to mucosa-binding molecules such as CT or CTB has been proposed as a strategy to induce local and systemic immune responses rather than systemic tolerance (23, 25, 34, 35).

In this study, oral administration of prototype antigens conjugated to CTB, rather than inducing systemic immune responses, dramatically enhanced induction of peripheral tolerance to these antigens. DTH and lymphocyte proliferative responses were markedly reduced in mice fed single doses of CTB-conjugated antigen. Serum antibody responses to the conjugated antigen were also decreased, although this effect was less pronounced than the suppression of DTH and also varied with the antigen tested. In contrast, oral delivery of unconjugated antigen administered in single or multiple doses required massive quantities of antigen to suppress late DTH reactivity and failed to affect the early DTH response. Since free CTB had no effect on systemic DTH reactivity to the co-fed antigen, the physical association of CTB and antigen is required to mediate such tolerogenic effects. Moreover, free CT but not CTB abrogated oral tolerance when coadministered with CTB-conjugated antigen, an observation which is in keeping with earlier findings on humoral immune responses (24) and extend them to cell-mediated (DTH) responses. The striking differences observed in this study between CT and CTB may explain why previous studies assessing the mucosal immunogenicity of various antigens coupled to CTB have not disclosed any suppression of systemic immune responses after mucosal administration of CTB-conjugated antigens. In those studies, CTB/antigen formulations have all contained small amounts of contaminating CT (23, 25) and/or CT had been added to potentiate the immunogenicity of CTB-antigen conjugates (34). By using a recombinantly produced CTB, inherently devoid of toxic activity, we have found that CTB can serve as a powerful carrier-enhancing agent for induction of peripheral immunological tolerance, a hitherto unrecognized property of this molecule.

Table 3. Oral administration of CTB-conjugated red cells induces suppression of systemic antibody responses in naive and in systemically immune mice

Oral tolerogen (day)†	Systemic immunogen	Geometric mean serum antibody titer (range)	
		IgM	IgG
CTB-SRBCs (−7)	SRBCs	320* (210–500)	3,840* (2,360–5,160)
SRBCs (−7)	SRBCs	940 (660–1520)	15,900 (2,000–21,200)
CTB-SRBCs (0)	SRBCs	660* (570–770)	4,700* (3,770–5,870)
SRBCs (0)	SRBCs	1020 (670–1580)	24,400 (21,700–27,400)
CTB-SRBCs (+4)	SRBCs	<50**	4,030* (2,820–5,770)
SRBCs (+4)	SRBCs	1500 (1230–1720)	23,300 (20,050–27,000)
Saline	SRBCs	1730 (1450–2020)	18,400 (4,080–29,000)
None	None	<50	<50

†Mice were fed single doses of CTB-conjugated or unconjugated SRBC at the indicated times.
‡Serum IgM and IgG antibody titers were determined on day 7 after systemic priming (day 0) and 5–7 days after footpad challenge (day +7), respectively. Data were calculated on sera collected from groups of six to eight mice and analyzed by direct (IgM) or indirect (IgG) hemagglutination assays for SRBCs. Significant differences with saline-fed animals are indicated (*, $P < 0.05$; **, $P < 0.01$; Wilcoxon rank test).

Table 4. CT abrogates CTB-induced suppression of systemic DTH

Exp.	Feeding*	Specific thickness increment†, cm × 10^3 (% inhibition)	
		2 hr	24 hr
1	CTB-SRBCs	−4 ± 4.5 (156; $P < 0.001$)	5 ± 2.4 (87; $P < 0.01$)
	CT-SRBCs	21 ± 4.1 (−133)	50 ± 5.6 (−28)
	Saline	9 ± 1.5	40 ± 2.9
2	CTB-SRBCs	−3 ± 2.0 (113; $P < 0.001$)	14 ± 6.5 (68; $P < 0.05$)
	CTB-SRBCs + 10 μg of CT	20 ± 3.9 (−18)	57 ± 7.8 (−30)
	CTB-SRBCs + 10 μg of CTB	−3 ± 3.1 (113; $P < 0.001$)	19 ± 8.2 (57; $P < 0.05$)
	CTB-SRBCs + 500 μg of CTB	0 ± 4.0 (100; $P < 0.001$)	11 ± 4.0 (75; $P < 0.01$)
	Saline	17 ± 4.2	44 ± 6.5

*Mice were fed single doses of CTB-conjugated SRBCs with or without free CT or CTB, given 7 days prior to systemic priming with SRBCs.

†Mean (+ SD) determined on groups of six to eight mice challenged 7 days after systemic priming. Where significant, differences between experimental and saline-fed control animals are indicated (Wilcoxon rank test).

In all instances, single oral administrations of CTB-linked antigens were effective at doses 15- to 500-fold lower than those of corresponding regimens using unconjugated antigens to suppress late DTH and serum antibody responses, and the suppression achieved was also more pronounced. Furthermore, this strategy of oral tolerance induction abrogated early DTH swelling reactions.

Previous studies have indicated that the classical 24-hr DTH skin reaction is preceded by an early swelling reaction that peaks 2–4 hr after challenge and can be transferred to naive recipients with sensitized T cells (30). In this study, suppression of DTH responses in mice fed a single dose of CTB-antigen lasted for at least 8 weeks and was manifest early (2–4 hr) as well as late (24–48 hr) after systemic challenge with antigen. In contrast, regimens involving repeated feeding of large doses of unconjugated antigen did not affect early DTH reactivity but were partially effective at suppressing the late DTH component. These observations suggest that the early and late components of DTH responses are differentially regulated. Consistent with this interpretation are the results of recent experiments in which suppression of early and late DTH reactivities in mice fed CTB-conjugated antigens could be transferred independently (unpublished results).

Further, feeding CTB-antigen suppressed systemic responses not only in naive animals but also in mice previously sensitized to the antigen, suggesting that memory cells are sensitive to the tolerogenic signals induced by mucosally delivered CTB conjugates. This finding is especially important since (*i*) oral tolerance, to be broadly applicable, must be effective in situations where potentially pathogenic lymphocytes exist and (*ii*) conventional oral tolerization protocols are less efficient at suppressing immune responses in systematically immune animals than in naive animals (19–22).

We have extended this finding to several other antigens, including autoantigens, alloantigens, and haptens, and have found that mucosal administration of minute amounts of CTB-conjugated antigen protects animals from autoimmune encephalomyelitis and contact allergy and can prolong survival of allografts (unpublished work).

Although much remains to be elucidated regarding the mechanisms governing induction of tolerance by mucosal administration of antigens linked to CTB (and conceivably also to other mucosal lectins) and the effectiveness in humans will have to be determined, this strategy may lead to the development of a class of agents to prevent and treat diseases caused by tissue-damaging immune responses.

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